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Ecomorphological Variation in Three Species of Cybotoid Anoles

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Abstract: Caribbean *Anolis* lizards exhibit a complex suite of ecological, morphological, and behavioral traits that allow their specialization to particular microhabitats. These microhabitat specialists, called ecomorphs, have independently evolved on the four islands of the Greater Antilles, and diversification among anole ecomorphs has been the focus of many studies. Yet, habitat specialization has also occurred among species within the same ecomorph group. Here, we examined ecological, morphological, and behavioral divergence in three Hispaniolan trunk–ground species, the cybotoid anoles: *Anolis cybotes, A. marcanoi,* and *A. longitibialis*. We found differences in limb morphology, locomotor behavior, and perch use among the three cybotoid species that mirror differences across the ecomorphs. Among these species of cybotoids, those that have longer limbs tend to move less frequently, occupy broader perches, and have smaller fourth toes with fewer lamellae. We also observed that the species with greater male-biased size dimorphism had larger heads, smaller dewlaps, and smaller testes. These results are consistent with the predictions of sexual selection theory, in that species with large male body size may have larger heads because of increased male–male combat, and smaller testes potentially attributable to a trade-off between pre- and postcopulatory selection. Overall, our study suggests that a combination of local adaptation to different structural habitats and sexual selection might produce ecomorphological diversification within cybotoid anoles of the same ecomorph group.

Key words: *Anolis*; Dewlap; Hindlimb; Locomotor behavior; Perch use; Sexual size dimorphism

Ecomorphology, the study of the relationship between an organism's ecology and its morphology, has revealed that evolution often shapes complex suites of traits to allow specialization to a particular environment. The evolution of such phenotypic suites has facilitated divergence into diverse habitats, driving adaptive radiations in multiple taxa (reviewed in Schluter 2000). In some groups, ecomorphological evolution has produced strikingly repeatable results, resulting in discrete classes of species adapted to a set of niches (e.g., *Eleutherodactylus* frogs, Hedges 1989; *Anolis* lizards, Losos et al. 1998; cichlid fish, Danley and Kocher 2001; boas and pythons, Esquerré and Keogh 2016). When habitat specialization in multiple, independent lineages results in the convergent evolution of ecology, morphology, and behavior, the resulting classes are termed "ecomorphs" (following Williams 1972), defined as specialists to a particular microhabitat. Although the evolution of ecomorphs has been a focus of many studies (e.g., Schluter 2000), divergence within an ecomorph, which can result in further habitat specialization (Losos 1996), has received far less attention.

Of the approximately 150 species of anole lizards (genus *Anolis*) in the Caribbean, most are categorized as one of six ecomorphs: trunk–ground, trunk–crown, trunk, grass–bush, twig, and crown giant (Williams 1972, 1983; Losos 2009). Specific ecomorphs have independently evolved on each of the islands of the Greater Antilles (Losos et al. 1998), and species within an ecomorph exhibit coordinated combinations of adaptations to their structural microhabitat. For example, trunk–ground species are generally medium-sized (male snout–vent length [SVL] from 50 to 75 mm), brown lizards with long limbs and a stocky build. As sit-and-wait predators, they perch low on tree trunks and forage on the ground and in low vegetation. On the other hand, twig species are mottled gray or brown, have short limbs and narrow bodies, and move slowly and cryptically on small branches in the canopy. There is a rich body of literature that compares a wide range of traits both among and within the anole ecomorphs (reviewed in Losos 2009; Wollenberg et al. 2013; Kamath and Losos 2017), but the majority of studies about within-ecomorph variation has focused on variation in traits not directly associated with anole ecomorphology (e.g., thermal ecology, Ruibal 1961; Rand 1964; Hertz et al. 2013; body size, Muñoz et al. 2014). In this study, we examined variation in the morphological, ecological, and behavioral traits that distinguish different ecomorphs within several species of a single ecomorph: the Hispaniolan trunk–ground anoles.

These closely related anoles, termed the "cybotoids," have spread into different macrohabitats across the Dominican Republic and Haiti. Thus, this clade offers an ideal opportunity to examine patterns of diversification within an ecomorph (Glor et al. 2003; Wollenberg et al. 2013). The group of cybotoid lizards includes *A. cybotes* (including *A. armouri* and *A. shrevei,* which are phylogenetically nested within the *A. cybotes* group; Wollenberg et al. 2013), *A. marcanoi, A. whitemani, A. longitibialis,* and *A. strahmi*. Here, we focus on three of these species (Fig. 1): *A. cybotes,* a macrohabitat generalist that occurs throughout the island of Hispaniola, generally at elevations below 1800 m; *A. longitibialis,* a species restricted to rocky outcrops in the xeric southwestern Dominican Republic (Gifford et al. 2003); and *A. marcanoi,* a species that occurs in south-central Dominican Republic in semixeric to semimesic forest habitats (Glor et al. 2003). Using these three species, we tested for differences in ecological, behavioral, and morphological traits among species of cybotoid anoles, and compared these patterns with the known patterns of among-ecomorph diversification.

One of the defining traits of anole ecomorphs is based on the relationship between limb morphology and perch

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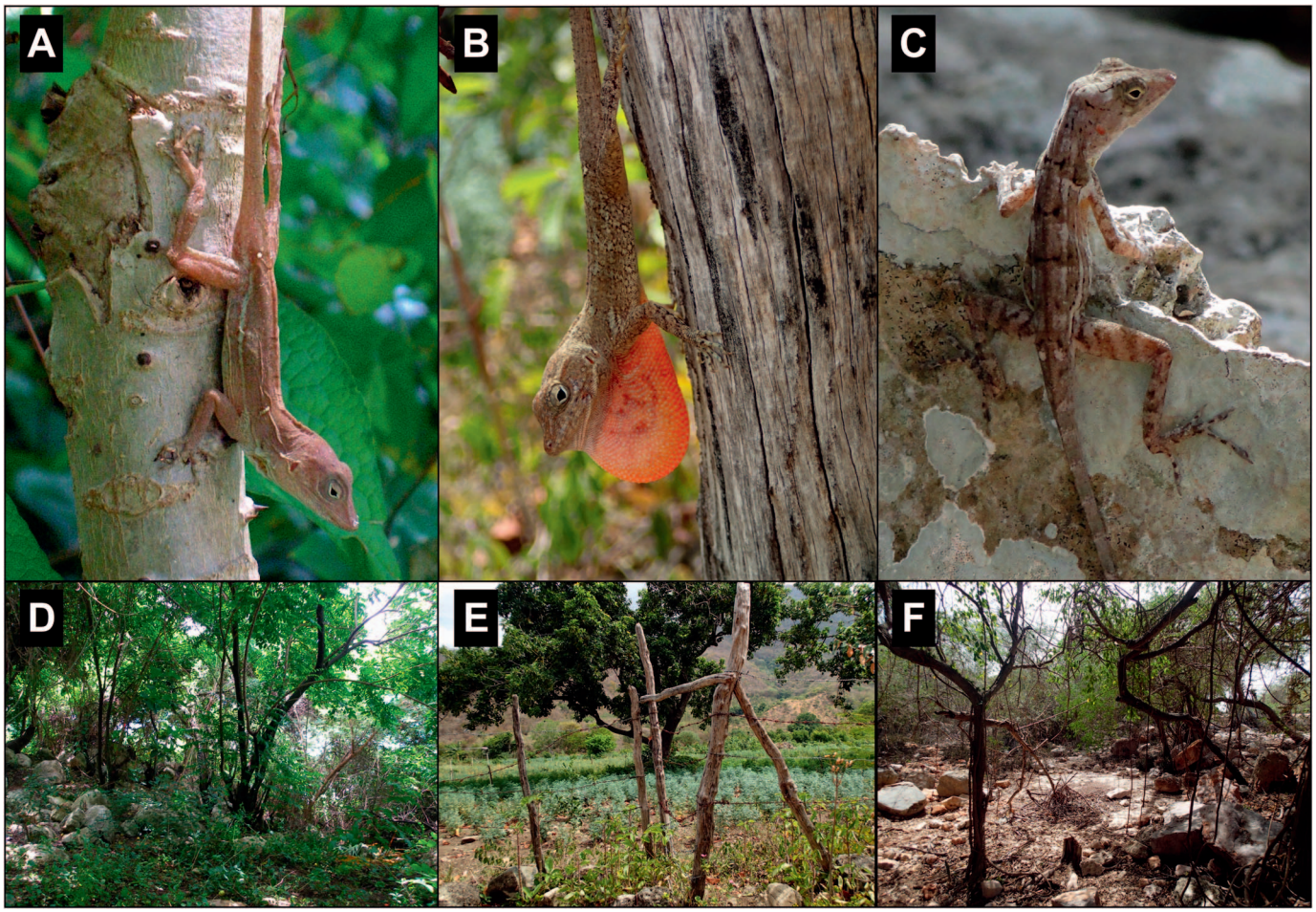

FIG. 1.—Photographs of adult male *Anolis cybotes* (A), *A. marcanoi* (B), and *A. longitibialis* (C), and their respective representative habitats: La Ciénaga, Barahona (D), the road to El Matedero north of Bani (E), and Manuel Goya (F) in the Dominican Republic. A color version of this figure is available online.

diameter. Longer-limbed species (i.e., trunk–ground and trunk–crown species) predominantly use larger or broader perches and exhibit greater sprint speeds on broad perches than shorter-limbed species (i.e., grass–bush and twig species; Losos 1990; Irschick and Losos 1999), whereas shorter limbs are generally associated with increased maneuverability on smaller perches (Losos and Sinervo 1989; Irschick and Losos 1998). In addition, toepad morphology is associated with clinging ability in anoles (Irschick et al. 1996), and the more arboreal anole ecomorphs generally have larger subdigital toepads, with more adhesive lamellae, than the more terrestrial ecomorphs (Glossip and Losos 1997). Locomotor performance in anoles is also dependent on substrate type (smooth or rough) and angle of incline, with lizards running faster on rough substrates and larger males pausing or slipping more frequently on smooth, vertical substrates (Kolbe et al. 2015). Furthermore, anoles preferentially use perches that allow maximum locomotor performance (Irschick and Losos 1999; but see Kolbe et al. 2015). Based on these findings, we tested three predictions regarding limb morphology, perch use, and locomotor behavior: (1a) Species with longer limbs, larger toepads, and more lamellae on their toepads will use broader perches. (1b) Longer-limbed species will perform more fast locomotor movements (i.e., runs and jumps), and shorter-limbed species will perform more slow movements (i.e., crawls). (1c) The macrohabitat generalist species (*A. cybotes*) will exhibit more variation in perch use and locomotor behavior, than the two macrohabitat specialist species (*A. longitibialis* and *A. marcanoi*). Although a range of perch diameters might be available in each habitat, the variation of perches available in the specialist habitats may be reduced, especially in xeric habitats.

Anole ecomorphs also differ in the extent of sexual size dimorphism (SSD), with trunk–ground and trunk–crown species exhibiting relatively high male-biased SSD, and the other four ecomorphs exhibiting relatively low SSD (Butler et al. 2000; Cox et al. 2007). This variation in SSD has been attributed to both ecological variation between the sexes and intrasexual selection (Butler et al. 2000; Cox et al. 2007). Here, we use SSD as a proxy for the strength of precopulatory sexual selection, following the empirical evidence that males in general, and particularly in lizards, evolve larger body sizes as the result of competition for mates or other resources (e.g., Andersson 1994; Cox et al. 2003, 2007). We tested for differences in sexually selected morphologies and behaviors, with the following predictions. (2a) Intense male conflict in anoles might escalate to locking jaws (e.g., Greenberg and Noble 1944; Jenssen et al. 2000); therefore, species with high SSD should exhibit relatively

larger head dimensions, which offer stronger bite forces (e.g., Herrel et al. 1999, 2001). (2b) Male anoles perform displays that include extensions of the dewlap (a colorful throat fan), head-bobs, and push-ups in courtship and aggression (Jenssen 1977). Thus, we predicted that species with high SSD will have larger dewlaps and more frequent dewlap and push-up display behaviors. (2c) Finally, because precopulatory male competition might be associated with an energetic trade-off with postcopulatory sperm competition (Fitzpatrick et al. 2012; Parker et al. 2013; Lüpold et al. 2014; Kahrl et al. 2016), we predicted that species with high SSD will have smaller testes.

MATERIALS AND METHODS

Measures of Behavior and Habitat Use

We examined adult male anoles of the three focal species in the Dominican Republic during the summer breeding season in 2005, 2009, 2010, and 2015. We studied *Anolis cybotes* at Coralsol Beach Resort in La Ciénaga, Barahona (18°03′42.2″N, –71°06′39.9″W; in all cases, datum = WGS84); *A. longitibialis* in Manuel Goya (17°50′8.5″N, –71°27′0.5″W and 17°48′9.216″N, –71°26′51.792″W); and *A. marcanoi* near El Matadero (18°23′10.0″N, –70°26′31.4″W; 18°24′9.936″N, –70°25′12.792″W; and 18°19′30.18″N, –70°17′20.508″W). We performed behavioral observations and morphological measurements in 2015, and recorded habitat use in each of the 4 yr of study.

We conducted focal observations on adult males of each species for 12–120 min/individual (*A. cybotes,* $n = 43$, 20.6 h observation; *A. longitibialis,* $n = 31$, 33.5 h observation; *A. marcanoi,* $n = 4$, 6.5 h observation), as follows. We located undisturbed lizards by walking slowly through the habitat, and then observed the behavior of the focal lizard using binoculars, from a minimum distance of 5 m. During behavioral data collection, we recorded all display behaviors (focusing on dewlap extension and head-bob and push-up displays), locomotor movements (with each movement categorized as a run, crawl, or jump), and copulations. To prevent repeated observations of the same male, we only observed lizards on perches separated by a minimum of 10 m, and we systematically conducted observations in different areas of the locality each day. All observations were performed between 0930 h and 1730 h, and never in inclement weather (i.e., rain). For each individual, we calculated rates of each display behavior (dewlap extensions, combined head-bobs and push-ups) per min, the total rate of locomotor movements per minute, and the average duration of each dewlap extension in seconds. We also determined the proportion of movements of each locomotor type (runs, crawls, and jumps) and the proportion of fast (runs or jumps) and slow (crawl) movements for each individual.

To obtain measures of habitat use, we noted the type of substrate on which each individual perched during behavioral observations. Perch diameter was collected for lizards that were seen before they were disturbed, and never for lizards that were first seen moving. Additionally, we recorded the orientation of the lizard on the perch as quasi-horizontal (0–30°) or quasi-vertical (31–90°).

Morphological Measurements

To collect morphological measurements, we captured 20 male and 20 female *A. cybotes,* 17 male and 20 female *A. longitibialis,* and 12 male and 11 female *A. marcanoi,* by hand or noose (these males were not always the same males on which behavioral observations were performed and perch data were collected). We measured the snout–vent length (SVL) of each lizard to the nearest mm using a plastic ruler. We calculated the average male and female SVL for each species, which was used to calculate the SSD of each species as

$$\text{SSD} = \frac{\text{Male SVL}}{\text{Female SVL}} - 1$$

following Lovich and Gibbons (1992). If males are larger than females, then by convention this measure of SSD is positive.

We measured a series of additional morphological traits in the adult males. Mass was measured using a Pesola spring scale (to the nearest 0.1 g), and external head and limb dimensions were each measured using digital calipers (to the nearest 0.1 mm; Mitutoyo, Japan). Head length was measured as the distance from the parietal eye to the tip of the snout, head width was measured at the widest part of the skull (the anterior base of the cranium), and head depth was measured at the deepest part of the skull (immediately behind the eyes).We measured the femur length as the distance between the body wall and the most distal point of the knee; tibia length as the distance between the knee and the most distal point of the foot; and fourth toe length as the distance between the tip of the longest toe (Metatarsal IV) not including the claw, and the point of insertion of the toe at the footpad. Three characteristics of the subdigital toepad on the fourth toe were also measured: number of lamellae on the toepad, pad length, and pad width. These traits were measured using digital flatbed scans with a resolution of 2400–3200 dots per inch, and were analyzed with the software ImageJ (NIH, USA). All foot measurements were taken twice, and we used the mean of these measures in subsequent analyses (modified after Zani 2000). We measured the hindlimb, fourth toe, and toepad on the right side of the lizard unless it was injured, in which case we measured the left side.

To measure dewlap size, we held the lizard's head parallel to a background of white graph paper and photographed the dewlap, fully extended using forceps, twice for each animal. We measured the area of both photographs of each dewlap using ImageJ, and then used the larger of the two measures in subsequent analyses.

These animals were also used in a series of other physiological studies, so a subset of lizards on which morphological measurements were made were transported to Trinity University and euthanized by rapid decapitation. Immediately following euthanasia, we opened the body cavity and measured the length and width of the right testis, before the testis was removed from the specimen. These measures were used to calculate testis volume using the formula for the volume of an ellipsoid ($4/3\pi a^2 b$), where a is the radius of the width of the testis and b is the radius of its length. We converted this measure of testis volume to a measure of mass using the density of testis tissue (1 mm^3/mg)

TABLE 1.—Results from separate analyses of variance testing for differences in $\log_{10}$-transformed body size (SVL), Head Size PC, and body size corrected morphology (residuals of morphological traits regressed against SVL) among *Anolis cybotes*, *A. longitibialis*, and *A. marcanoi*. Statistically significant comparisons are indicated with an asterisk (*).

Trait	$F_{2,39}$	P
Log_{10} SVL	12.24	< 0.001*
Residual mass	5.33	0.01*
Residual head length	2.86	0.07
Residual head width	0.35	0.74
Residual head depth	0.33	0.72
Head size PC	8.15	0.001*
Residual femur length	16.16	< 0.001*
Residual tibia length	4.66	0.02*
Residual fourth toe	9.47	< 0.001*
Residual toepad length	2.12	0.16
Residual toepad width	3.30	0.07
Number of lamellae	5.56	0.02*
Residual dewlap size	11.89	0.001*

reported by Licht and Pearson (1969). We then calculated gonadosomatic index (GSI, the ratio of testis mass to body mass) for each individual.

Statistical Analysis

We conducted all statistical analyses in JMP (v9.0, 2010; SAS Institute Inc., USA). All morphological traits were $\log_{10}$-transformed for our analyses. Head measurements are highly correlated, so we used Principal Component Analysis (PCA) to reduce dimensionality of $\log_{10}$ head length, $\log_{10}$ head width, and $\log_{10}$ head depth. This returned one significant PC ($\chi^2 = 135.34$, df = 5, $P < 0.001$) that explained 92.6% of the variance and had positive loadings of head length (0.95), depth (0.96), and width (0.97). We interpreted this PC as an overall measure of head size, and included this as Head Size PC in subsequent analyses.

To test for differences among species in body shape, we used body size-corrected residuals from the regression of the $\log_{10}$-transformed trait on $\log_{10}$ SVL, which included all males of all species. We then used a series of analyses of variance (ANOVA) to test for differences among the three species in $\log_{10}$ SVL, Head Size PC, GSI, number of lamellae on the fourth toepad, and the residuals of mass, head length, head width, head depth, femur length, tibia length, fourth toe length, fourth toepad length and width, and dewlap size. We used Tukey's Honest Significant Difference post hoc tests for all pair-wise comparisons following significant ANOVA results.

We used a series of ANOVAs to test for differences among species in the following dependent variables: rates of behavioral traits (dewlap extension, head-bobs and push-ups, total movements), proportion of type of locomotor movements (runs, crawls, or jumps) as well as fast (runs and jumps) and slow (crawls) movements, and the average duration of dewlap extension among the species. Copulation was rarely observed during these observations (in total, we saw only one pair of *A. cybotes* copulate during this study), and thus this behavior could not be statistically analyzed. We used a series of Brown–Forsythe tests to assess the equality of variance in behavioral traits among the species. We also tested for differences in substrate use and substrate orientation (quasi-horizontal or quasi-vertical) between species using χ^2 tests. We compared differences in perch diameter between *A. cybotes* and *A. marcanoi* using ANOVA. Our data on perch diameter for *A. longitibialis* were very limited; therefore, we removed *A. longitibialis* from the analysis of perch diameter.

RESULTS

Morphological analysis showed that the three cybotoid species differed in body size and limb dimensions (Table 1; Fig. 2; Table S1 in the Supplemental Materials available online). *Anolis cybotes* and *A. longitibialis* did not differ in SVL, but *A. cybotes* had the largest relative mass, whereas *A. marcanoi* was the smallest species in both measures of overall size. We also found that *A. longitibialis* had longer hindlimbs and shorter fourth toes with fewer lamellae than *A. cybotes* and *A. marcanoi,* and *A. cybotes* had shorter hindlimbs, longer toes, and more lamellae than the other species (Table 1; Fig. 2).

In addition, we found that the species differed in locomotor behavior and perch type (Fig. 3; Table S2 in the Supplemental Materials available online). *Anolis cybotes* moved more frequently than *A. longitibialis* ($F_{2,49} = 6.23$, $P = 0.003$; Fig. 3), and had a greater variation in the frequency of their movements ($F_{2,75} = 6.83$, $P = 0.001$). However, the proportion of each type of movement (runs, crawls, and jumps) did not differ among the three species (all $P > 0.17$; Fig. 3), nor did the proportion of fast movements (runs and jumps; $F_{2,69} = 0.10$, $P = 0.25$), or slow movements (crawls; $F_{2,69} = 2.38$, $P = 0.10$). The variance of each of these types of movements was similar among all three species (all $P > 0.18$).

Perch use differed among the species ($\chi^2 = 122.9$, df = 10, $P < 0.001$); *A. longitibialis* was most often found on rocks, while *A. marcanoi* was most often found on fence posts and tree trunks. *Anolis cybotes* occupied the widest range of habitats, but was predominantly found on tree trunks and branches (Fig. 3), and was found on vertical branches more often than *A. marcanoi* and *A. longitibialis* ($\chi^2 = 13.05$, df = 2, $P = 0.001$). We found no difference in perch diameter between *A. cybotes* and *A. marcanoi* ($F_{1,26} = 3.06$, $P = 0.09$).

The three species also differed in the extent of SSD, with *A. longitibialis* (SSD = 0.352) exhibiting the most male-biased dimorphism, followed by *A. cybotes* (SSD = 0.169), and *A. marcanoi* (SSD = 0.085). Consistent with our predictions, the species with smallest SSD (*A. marcanoi*) had the smallest heads (Head Size PC; Fig. 4A), but it exhibited the largest relative dewlaps (Table 1; Fig. 4D). Despite differences in the Head Size PC, the three species were similar in head length, width, and depth (Table 1). Furthermore, our prediction that other morphological and behavioral traits associated with precopulatory sexual selection would show a directional pattern with the extent of SSD was not supported. *Anolis cybotes,* the species with intermediate SSD, performed a higher rate of dewlap extensions (Fig. 4E; $F_{2,77} = 12.20$, $P < 0.001$) and head-bobs/push-ups (Fig. 4C; $F_{2,77} = 13.31$, $P < 0.001$) than *A. longitibialis*. The average duration of dewlap extension was similar between the two species (Fig. 4F; $F_{2,59} = 2.18$, $P = 0.122$). Testis morphology differed among the three species, with the least dimorphic species (*A. marcanoi*) having a larger GSI than the two more dimorphic species (Fig. 4B; $F_{2,49} = 6.23$, $P = 0.003$).

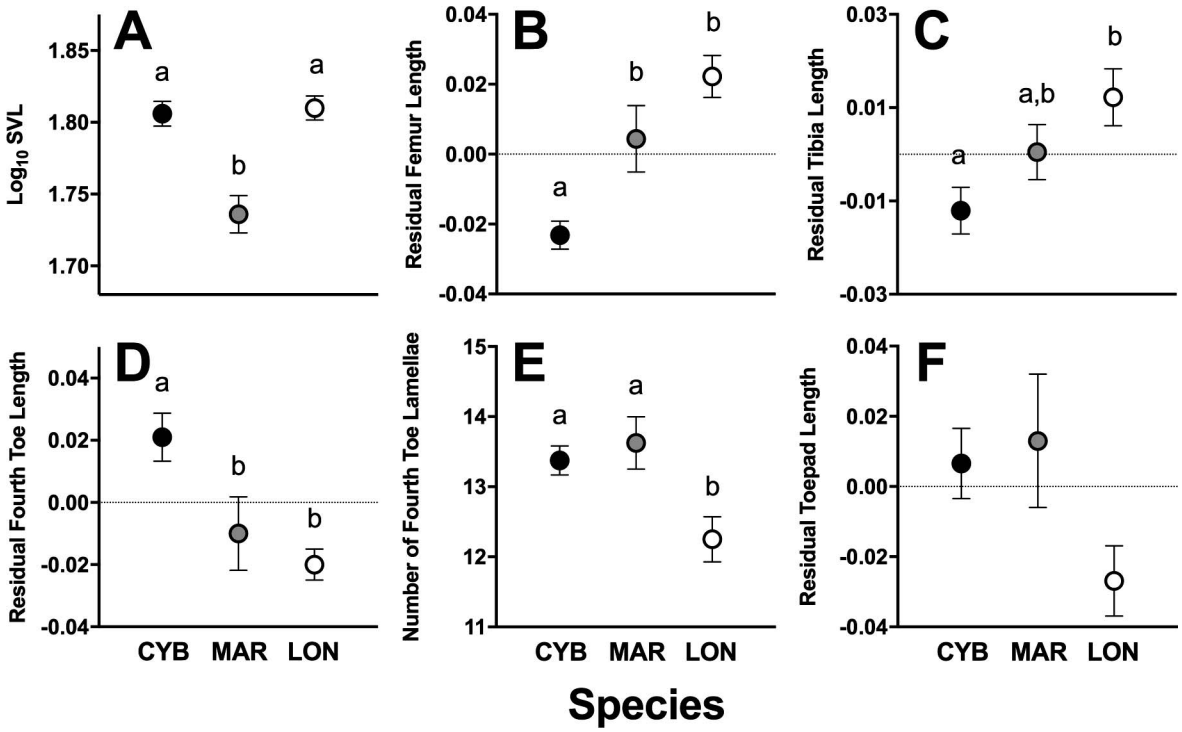

FIG. 2.—Means ± 1 SE of $\log_{10}$ snout–vent length (SVL; A), residual femur length (B), residual tibia length (C), residual fourth toe length (D), number of lamellae on the fourth hindlimb toe (E), and residual fourth toepad length (F) for *Anolis cybotes* (CYB; $n = 17$), *A. marcanoi* (MAR; $n = 7$) and *A. longitibialis* (LON; $n = 17$). Superscripts denote differences between species ($P < 0.05$).

DISCUSSION

Caribbean anoles are a classic example of adaptive radiation via niche partitioning, given that ecomorphs have evolved to occupy a wide range of microhabitats (Williams 1983; Losos 2009). Yet even within the ecomorph groups, several clades have undergone further diversification within their particular microhabitat on a given island (Burnell and Hedges 1990; Glor et al. 2003, 2004; Knouft et al. 2006; Wollenberg et al. 2013). The cybotoid anoles provide a model for studying local adaptation and speciation within a clade because this group occupies a wide range of habitats on Hispaniola, and exhibits morphological differences that could be attributed to this ecological variation. We have demonstrated differences in the morphology, ecology, and behavior of three species of closely related cybotoid anoles. Consistent with our predictions, the differences in substrate use, morphology, and behavior within this ecomorph mirror some patterns of diversification among other anole ecomorphs, and among other species of lizards. This indicates that each cybotoid species has experienced local adaptation to the variable habitats in the Dominican Republic. For example, among anole ecomorphs, species that use broader perches tend to have longer limbs (Losos and Sinervo 1989;

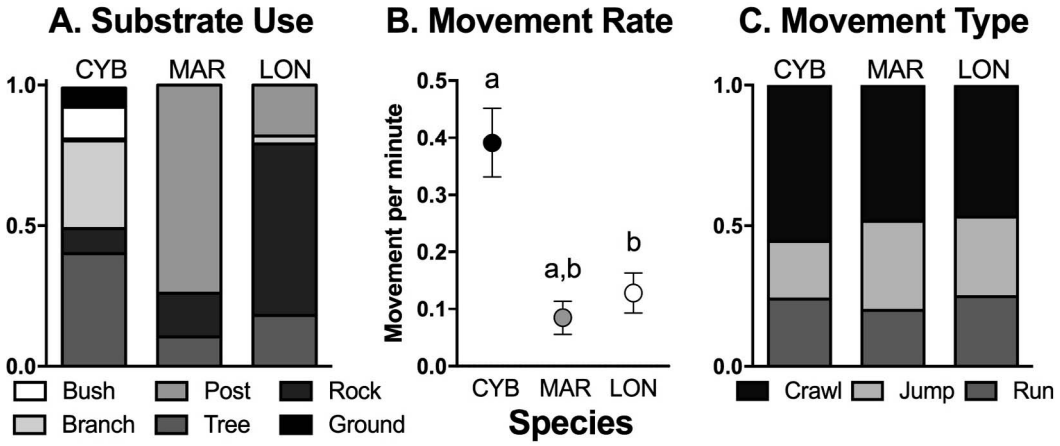

FIG. 3.—Comparisons of ecology (substrate use, A), and behavior (mean ± 1 SE of the movement rate, B, and movement type, C) for *Anolis cybotes* (CYB), *A. marcanoi* (MAR), and *A. longitibialis* (LON). Substrate use (A) is graphed as a proportion of total observation. Movement rate (B) was calculated as the total number of movements (run, crawl, and jump) per minute. Movement type (C) is expressed as a proportion of the total movements observed for each species.

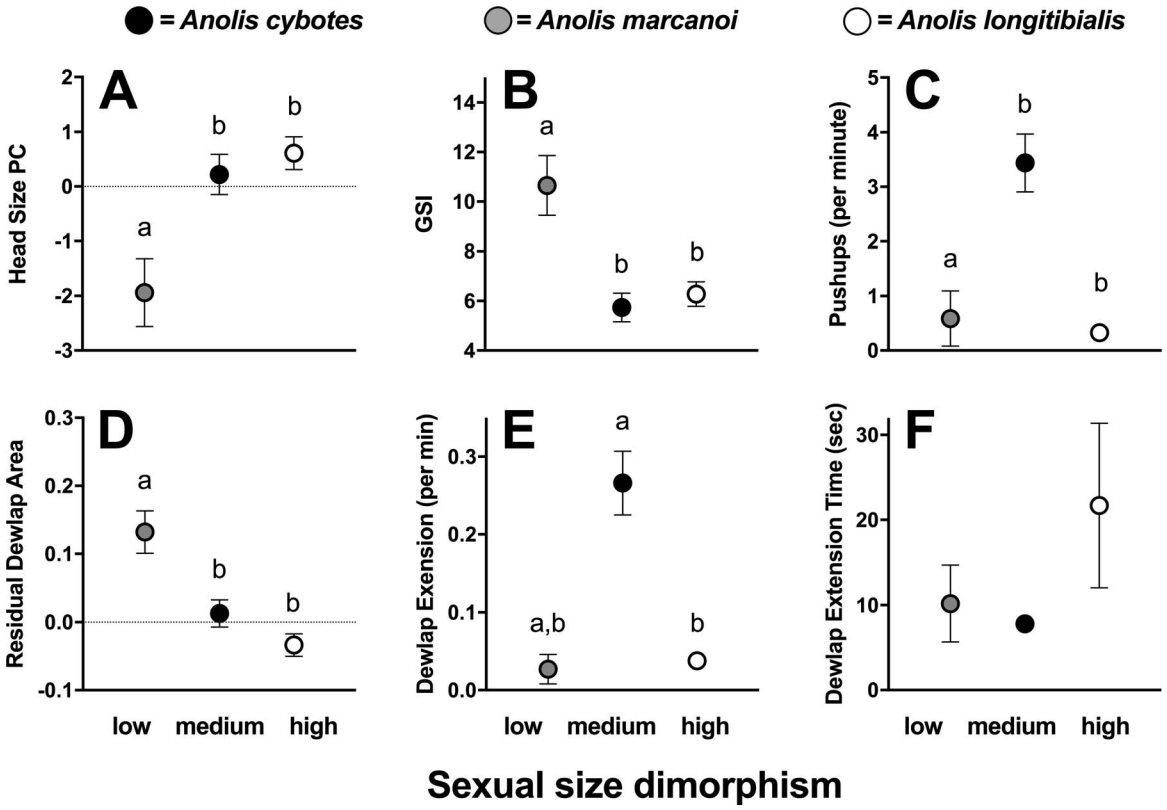

FIG. 4.—Comparisons of sexual size dimorphism (SSD) and other sexually selected traits in cybotoid anoles. Means ± 1 SE of the principal component representing of lizard head size (Head Size PC; A), gonadosomatic index (GSI; B), push-ups per minute (C), residual dewlap area (D), dewlap extensions per minute (E), and dewlap extension time (F) for *Anolis cybotes, A. marcanoi,* and *A. longitibialis*. These data are presented in order of increasing extent of sexual size dimorphism in each species: *A. marcanoi* (SSD = 0.085), *A. cybotes* (SSD = 0.169), and *A. longitibialis* (SSD = 0.352).

Losos 1990). Our data also indicate that species with low male-biased dimorphism tend to have smaller heads and larger testes, consistent with theory on sexually selected traits in this group (Kahrl et al. 2016). We found no relationship, however, between SSD and social display behaviors. Although we found differences in these traits among species, we could not directly test for associations between morphology, ecology, and behavior because we only examined these traits in three species of anoles. However, these data provide one of the first comparisons of behavior and sexually selected traits in this group.

Morphology and Substrate Use

Cybotoid anoles are a morphologically diverse group that inhabit a range of perch substrates and macrohabitats in the Dominican Republic (Glor et al. 2003). Accordingly, we hypothesized that variation in microhabitat or substrate use might have led to predicable changes in morphology and behavior, similar to the patterns of ecomorphological divergence across Caribbean anoles (Losos and Sinervo 1989; Losos 1990). For the three species examined here, we found that the species using the narrowest perches also has shorter hindlimbs, and longer fourth toes with more lamellae on the toepad (*Anolis cybotes;* Figs. 2, 3). This result is consistent with patterns of adaption among species across ecomorphs, where species that live on smaller perches tend to have shorter limbs with more lamellae, traits thought to aid in clinging ability while running on small perches (Cartmill 1985; Pounds 1988; Losos 1990). Our results also corroborate previous research showing deterministic evolution in morphology of *A. cybotes,* which partially mirrored diversification in previous stages of this adaptive radiation (Wollenberg et al. 2013). Together, these traits indicate that this species has likely adapted to climbing and running on relatively narrow perches (Irschick et al. 1996; Zani 2000).

We also found that the morphology associated with living on rocks in *A. longitibialis* mimics the patterns of morphology seen in other species of lizards. In particular, the longer limbs of *A. longitibialis* might be an adaptation to its habitat that enable this species to run faster and jump farther on broad, rocky surfaces (Losos 1990; Irschick and Losos 1999). Similar morphological features occur in other saxicolous anoles, including *A. eugenegrahami* and *A. aquaticus,* who have elongated limbs and flattened bodies (Leal et al. 2002; Muñoz et al. 2015). This morphological adaptation is not limited to anoles, but is common among other groups of lizards. In fact, the evolution of long limbs, toes, and flattened bodies has been repeated in five other lineages of rock-dwelling lizards (Revell et al. 2007; Goodman et al. 2008), and is linked with improved performance in jumping, sprinting, and climbing on rocky habitats (Goodman et al. 2008). This indicates that the ecomorphological adaptations seen in anoles can serve as a general model for how other species of lizards might adapt to specialized habitats.

Species might exhibit population-level variation—especially those that, like the cybotoid anoles, exist across heterogeneous landscapes. Therefore, it is important to

recognize that there are limitations in using data from one or a few populations to represent a species. The data presented here are a snapshot of the true diversity in behavior, morphology, and substrate use in each species. In particular, we examined a lowland population of *A. cybotes* in this study, yet this species occurs in montane areas as well. Parallel to the ecomorphological associations among the three species reported in this study, *A. cybotes* exhibits similar ecomorphological associations in limb dimensions and microhabitat along the altitudinal clines of Hispaniola (Wollenberg et al. 2013; Muñoz et al. 2014). This intraspecific variation further supports the idea that local ecomorphological adaptation in cybotoids is widespread, both within and among each species.

Behavior and Substrate Use

Contrary to our hypothesis, we found no difference in the proportion of fast or slow movements between species of anoles. We did find, however, that the long-limbed saxicolous species (*A. longitibialis*) tended to move less frequently than the short-limbed tree-dwelling species (*A. cybotes*). This relationship between microhabitat use and movement rate again mimics relationships observed among older stages of the anole radiation, where species that live on tree trunks or branches tend to have greater movement rates than those that live near or on the ground (Johnson et al. 2008). This variation in movement rate might be caused by differences in foraging behavior among species, associated with variation in visibility from the lizard's perch (Johnson et al. 2008). In particular, microhabitats with a greater density of twigs and branches tend to obscure visibility, and lizards might move more frequently to survey the areas around them for prey or conspecifics (Moermond 1979).

We also tested the hypothesis that generalist species, which use more diverse substrates, exhibit greater variation in their locomotor behavior. *Anolis cybotes* used the widest variety of substrates, while both *A. marcanoi* and *A. longitibialis* were observed on fewer substrate types, and on a single substrate type >60% of the time (Fig. 3A). This difference in perch use might be a function of species-specific preferences for particular perches, perch availability, or an interaction of the two factors (Johnson et al. 2006; Herrmann 2017). Consistent with our hypothesis, we also found that individual *A. cybotes* had greater variation in their overall movement rate than members of the other two species. Because *A. cybotes* occupies more diverse perch types, there might be greater variation in visibility and therefore, high variation in movement rates in this species (Moermond 1979). We note that our behavioral observations of *A. marcanoi* were limited, however, and our species-level quantification of their behavior may be estimated with some error.

Sexually Selected Traits

We measured several traits associated with pre- and postcopulatory selection in each of these three species to examine patterns between SSD, morphology, and social behavior. Although we could not statistically test this relationship among species, we observed patterns consistent with hypotheses about how precopulatory selection might influence trait evolution among species. These species vary in the extent of male-biased sexual size dimorphism (Fig. 4), which can result from precopulatory selection on male body size (Butler et al. 2000; Cox et al. 2003, 2007). We predicted that species with high male-biased SSD would also have larger heads, and deeper and wider heads in particular because of the use of the head for biting during male–male combat (Lailvaux et al. 2004; Lailvaux and Irschick 2007). We found that the species with medium and high SSD (*A. longitibialis* and *A. cybotes*) had larger heads (Head Size PC) than the species with low SSD, *A. marcanoi* (Fig. 4A). Whereas lizard bite force or head size can predict the outcomes of male–male combat (e.g., Hews 1990; Pratt et al. 1992; López et al. 2002; Gvozdík and Van Damme 2003; Husak et al. 2006), little comparative work in squamates has tested the hypothesis that species with high male-biased SSD also have positive allometry for head size (Carothers 1984). Our study suggests a positive association between SSD and head size, but many more species should be sampled to test this hypothesis.

We also predicted that species with high male-biased SSD would exhibit larger dewlaps and higher display rates, but we found that the species with the highest male-biased SSD had the smallest dewlap area (Fig. 4). We also found no similarity between SSD and display rate in these species (Fig. 4). A variety of hypotheses have been proposed to explain variation in dewlap size and patterning among species of anoles (Williams and Rand 1977; Losos and Chu 1998; Leal and Fleishman 2004; Nicholson et al. 2007). Many tests of these hypotheses have found weak or no support for differences among ecomorph groups or for variation attributable to species recognition (Nicholson et al. 2007). Among islands, anole species that co-occur with other species of anoles did not differ in their dewlaps when other cues for species recognition were present (i.e., when there were strong differences in other phenotypic traits). In populations where other cues were lacking, however, anoles tended to have more variable dewlap colors (Williams and Rand 1977). Among the three populations that we sampled, *A. marcanoi* had reddish-pink dewlaps, whereas both *A. cybotes* and *A. longitibialis* had pale yellow or white dewlaps. Although our data cannot test this hypothesis, these observed differences in dewlap size and color might aid in species-recognition, especially given the physical similarities between these species. Whereas it was not a focus of our study, dewlap size is also weakly associated with habitat illumination, which might contribute to the evolution of the dewlap (Losos and Chu 1998; Leal and Fleishman 2004).

We observed that species with higher male-biased SSD had smaller testes (Fig. 4B). This pattern was consistent with our predictions of a trade-off between pre- and postcopulatory selection, and mirrors a trade-off that exists both among other species of anoles and across all squamates (Lailvaux et al. 2004; Lailvaux and Irschick 2007; Kahrl et al. 2016). This indicates that the patterns of diversification in morphology attributable to sexual selection that occur among species are also occurring within ecomorph groups.

Our results might inform patterns of divergence among other sympatric or allopatric anole species that exhibit niche partitioning or local adaptation, respectively, within the same ecomorph (Glor et al. 2003, 2004; Knouft et al. 2006), similar to the patterns observed among ecomorphs on each island (Williams 1983; Losos 1990; Losos et al. 1998). In particular, the cybotoid group of anoles show differences in morphology

that are independent of their phylogenetic relationships (Glor et al. 2003), which can be attributed to a combination of microhabitat and genetic differentiation between these species (Wollenberg et al. 2013). We found that closely related species—*A. cybotes, A. longitibialis,* and *A. marcanoi*—exhibited striking differences in their morphology, ecology, and behavior that might be caused by local adaptation to varying microhabitats and differences in the strength of sexual selection among species, which might represent a yet underexplored dimension of local adaptation.

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Supplemental Material

Supplemental material associated with this article can be found online at https://doi.org/10.1655/Herpetologica-D-17-00040.S1; https://doi.org/10.1655/Herpetologica-D-17-00040.S2.

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